Remarks

Independent claims 1 and 16 are amended to improve clarity and address the issues raised in the Office Action. Similarly, dependent claims 2, 3, 17, and 18 are amended to correspond better to the independent claims and improve clarity. No new matter is added by these amendments.

The rejection of claim 1-42 under 35 U.S.C. § 112, second paragraph

All pending claims are rejected as indefinite and unclear under 35 U.S.C. § 112, second paragraph. The rejection is respectfully traversed.

The Office Action states that the claims are "vague and confusing overall." The rejection states that the claims do not clearly state to what the primers and probes hybridize. The rejection states that the claims do not clearly state what constitutes the conserved regions and what constitutes the first through fifth divergent regions. The rejection states that the claim are not clear because no specific sequences have been identified. Each of these is addressed separately below.

The Office Action provides a separate section on claim interpretation. Because the applicants previously explained that "the claims do not require hybridization of the first or second probe to *S. aureus* or to *B. japonicum*" the examiner construes the recited conserved and divergent regions as representing ***any*** sequences found in the 16S rRNA gene of eubacteria species. Applicants believe that its statement has been misunderstood. Nonetheless, to avoid doubt, the term "hybridizes" has been stricken from the claims and replaced with "complementary." This change emphasizes that a property of the primer is being described, *i.e.,* capable of hybridizing due to complementarity, rather than an active step of hybridization that must occur as part of the claimed method.

- To what do the primers and probes hybridize?

The primers are complementary to two flanking regions of a *S. aureus* 16S rRNA gene. The flanking regions flank a segment of the *S. aureus* 16S rRNA gene that comprises a conserved region and a divergent region. Thus the primers would hybridize to *S. aureus* 16S rRNA if it were present. It will also hybridize to other eubacteria that also have these flanking regions. As stated at page 6, [20], the PCR primers "prime virtually universally across species of eubacterial 16S rRNA genes." See also page 7, [22]. Exemplary primers are shown in Figure 5, first and fourth column of sequences. Because the primers must hybridize in order to prime, they have a complementary sequence to the *S. aureus* 16S rRNA. They are also highly conserved in order to prime virtually universally across species of eubacterial 16S rRNA genes.

- What constitutes the conserved regions?

The conserved regions comprise at least 18 contiguous nucleotides which are at least 80 % identical among at least 10 eubacterial species. See Figure 5, third column of sequences.

- What constitutes the first through fifth divergent regions?

The first divergent region comprises at least 10 contiguous nucleotides of a *S. aureus* 16S rRNA gene and differs by at least 3 nucleotides from a divergent region found in a *Bradyrhizobium japonicum* 16S rRNA gene. See Fig. 5, second column of sequences. The first divergent region is complementary to the first fluorogenic probe.

The second divergent region is found in *Bradyrhizobium japonicum* 16S rRNA gene. See GenBank accession numbers D12781, X87272. and X71840.

The third divergent region is in the 16S rRNA gene of the first species of eubacteria, *i.e.,* the species that is in the test sample and which is detected by the second probe.

The fourth divergent region is in the 16S rRNA gene of a second species of eubacteria which may be present in the test sample. The fourth divergent region is complementary to the third fluorogenic probe.

The fifth divergent region is in the 16S rRNA gene of a third species of eubacteria which may be present in the test sample. The fifth divergent region is complementary to the fourth fluorogenic probe.

- Have any specific sequences been identified?

Indeed, the inventors have disclosed flanking regions, and conserved and divergent regions for each of 15 eubacterial species. See Figure 5. As displayed graphically, the relative positions of the flanking regions to the conserved and divergent regions is relatively fixed. One flanking region is 5' and one flanking region is 3' of the segment comprising both a conserved and a divergent region. The high degree of sequence conservation among the flanking regions and among the conserved regions is shown. The high degree of sequence divergence among the divergent regions is shown.

It is respectfully submitted that the claims are clear (although complex) and if read with care and in light of the specification, yield answers to the questions posed.

The rejection of claims under § 103(a)

Various combinations of references are applied by the U.S. Patent and Trademark Office to assert that the claims are obvious. All combinations use primary references Greisen and Reischl. Griesen is cited as teaching all elements of the claims except the use of real-time PCR with fluorogenic probes. Reischl is cited as teaching that form of PCR. It is respectfully submitted that even if one of skill in the art were motivated to combine these two teachings, he would not have had a reasonable expectation of success. Moreover, none of the secondary references remedy that deficiency in the primary references.

Section 103(a) of 35 U.S.C. states:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.

Obviousness under 35 U.S.C. § 103(a) is a question of law based on several factual inquiries:

> Under § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at

> issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved.

Graham v. John Deere Co., 383 U.S. 1, 17 (1966). The U.S. Patent and Trademark Office bears the initial burden of establishing a *prima facie* case of obviousness based on the results of the factual inquiries under *Graham*. A *prima facie* case of obviousness has three elements:

> First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

Manual of Patent Examining Procedure, 8th ed., § 2142. In the present application, the results of the factual inquiries under *Graham v. John Deere Co.* do not support a *prima facie* case that any of the claims are obvious under 35 U.S.C. § 103(a) over any of the cited combinations of references.

Independent claim 1 recites "performing a real-time polymerase chain reaction (PCR) using a sample which may comprise template DNA of a first species of eubacteria, wherein the PCR employs primers and **at least two fluorogenic probes**." Independent claim 16 similarly recites a real-time PCR reaction mixture that comprises "at least two fluorogenic probes." The first probe is complementary to the conserved region of the amplified segment of the 16S rRNA and the second probe is complementary to the divergent region of the amplified segment of the 16S rRNA. The two regions are adjacent to each other within the segment of 16S rRNA gene.

In order for the claimed methods to be operative, the two probes must hybridize to the segment. As amplification proceeds, the probe is displaced from the template and subsequently degraded. See Exhibit 1. One of ordinary skill in the art could not have known from the

teachings of the cited references that real-time PCR would work properly when at least two probes must hybridize to the same amplified segment. These probes could have provided steric hindrance because they must hybridize to the same templates within the same amplicon. *A priori,* one of skill in the art would not have had a reasonable expectation of success.

This is even more the case for the method of claims 2, 3, 17 and 18 which require third and fourth probes in the reaction mixture. In these methods, each of the second, third, and fourth probes compete for the same stretch of template, *i.e.,* the divergent region. One of ordinary skill in the art could not have known that using multiple competing probes would permit the real-time PCR reaction to operate successfully.

Greisen is cited as teaching primers and probes for amplifying and detecting pathogenic bacteria using their 16S rRNA genes. Broad range primers and probes are used. Probes for detecting species or genera of particular contaminants of clinical samples were also used. Standard rather than real-time PCR was used. Southern hybridization was used as a means of detection.

Reischl is cited as teaching a real-time fluorescence PCR for the *mecA* gene and a genomic fragment of *S. aureus* that is described as a specific marker for *S. aureus*. This assay amplified two fragments from two different genes, the *mecA* gene and the *S. aureus*-specific genomic marker. See page 2430, second column. Two separate pairs of primers and two separate hybridization probes were used; two separate amplicons were formed ("The duplex approach containing four different primer oligonucleotides and four different hybridization probes within a single capillary,…"; page 2431, last sentence). Thus Reischl does not address whether one could successfully use the real-time PCR assay to successfully perform the methods of the present invention, using a single set of primers, a single amplicon, and multiple probes. Reischl does not teach whether this could be done using a eubacterial 16S rRNA gene, in particular. Moreover, Reischl provides support for the unpredictability that surrounded real-time PCR assays in general: "Significant formation of primer dimers or secondary structures or other cross-reactions between oligonucleotide components, which frequently interfere with the

analytical sensitivity of multiplex PCR approaches, are therefore unlikely in this particular assay." Page 2432, first sentence. Such uncertainties as posed by the use of multiple probes on a single amplicon are not even included within the concerns expressed by Reischl. Such uncertainties and potential for interference posed by the use of multiple probes that hybridize to the same divergent region of different species are also not included within the concerns expressed by Reischl.

Thus it is respectfully submitted that one of skill in the art would not have had a reasonable expectation of success even if they were, *arguendo,* motivated to try the present invention.

Moreover, Corless (2000) "Contamination and Sensitivity Issues with a Real-Time Universal 16S rRNA PCR," J. Clin. Microbiol. 38, 1747, already of record, teaches that there were technical issues evident with the type of approach that the present applicants ultimately employed. Corless concluded that without the development of various reagents and lab tools, "the implementation of a PCR for detection of eubacterial 16S rRNA with the TaqMan system [real-time PCR] will continue to be problematical." Abstract, last sentence. In fact, Corless was so discouraged by the technical problems, that he moved on to a system for the same purpose that did not use 16S rRNA genes at all. In 2001, Corless reported on a method that employed three different genes in a multiplex real-time PCR reaction. "Simultaneous Detection of *Neisseria meningitides, Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis an septicemia using real-time PCR," J. Clin. Microbiol. 39, 1553-1558; Exhibit 2. Corless used three different genes rather than use the single gene, single amplicon approach used in the claimed method. Corless's publications show that when confronted with the technical hurdles faced also by the present inventors, Corless (one of skill in the art) took a different approach. The approach of the present inventors was not so obvious that Corless selected it, even given the prior art cited by the Patent Office. These publications provide a picture of the state of the art around the time of the invention. Given the state of the art, the solution found by the present applicants was not obvious.

The secondary references do not cure the deficiencies of the primary references. Abrams, Iverson, Buck, Kunsch, and Barry are cited for teachings regarding issues ancillary to the main one outlined above.

- Abrams and Iverson are cited as teaching sequences similar to particular primer sequences of SEQ ID NO: 1 and 2. The existence of these primers does not in any sense obviate the non-obviousness of the claimed methods as a whole.
- Buck relates to the design of primers for sequencing. Sequencing employs a totally different reaction from that involved in the presently claimed methods. Buck does not appear to use a real-time PCR reaction. Thus Buck's discussion of primers does not implicate the use of multiple probes for a single amplicon in a real-time PCR reaction.
- Kunsch is cited as teaching a similar sequence to SEQ ID NO: 3. Kunsch is cited for using the sequence to detect *S. aureus* strains. This teaching does not remedy or cure the defects of the primary references as teaching the claimed method for detecting eubacteria and determining their species.
- Barry is cited for teaching a sequence that aligns with SEQ ID NO: 4. Barry does not, however, appear to address the issue of multiple probes on a single amplicon in a real-time PCR reaction. Thus Barry does not cure the deficiencies of the primary references.

While relating to some aspects of claimed methods, the secondary references do not address the state of the art and the ideas current at the time of the invention. As discussed above, the present invention would not have been obvious to those of ordinary skill in the art because there were technical obstacles relating to the use of real-time PCR and 16S rRNA, as reported by Corless, 2000. No piece of cited prior art suggested the use of multiple probes on a single amplicon in a real-time PCR assay. Surprisingly, this assay successfully detects and distinguishes among similar bacteria, such as various species of the same genus. See Table 4 and Table 5.

Applicants request that all rejections be withdrawn and that the application be speedily allowed.

Respectfully submitted,

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